Original Article

Synergistic effects of ACE (I/D) and Apo E (Hha I) gene polymorphisms on obesity, fat mass, and blood glucose level among the adult Asian Indians: A population-based study from Calcutta, India

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ABSTRACT

The study was aimed to determine the association of angiotensin converting enzyme (ACE) insertion/deletion (I/D) and apolipoprotein E (Apo E) *Hha I* gene polymorphisms with obesity, fat mass, and blood glucose levels in Asian Indian population. A total of 350 (184 men and 166 women) adult (30 years and above) Asian Indians of Calcutta and suburb participated in the study. Anthropometric measures, fat mass, and blood glucose measures were collected. Out of 350 subjects, a sample of 139 individuals was collected randomly for genotyping (adjusted for age and sex). The ACE and Apo E genotypes were determined by agarose gel electrophoresis. It was observed that neither ACE (I/D) nor Apo E (Hha I) gene polymorphisms showed any significant association with body mass index, waist circumference, fat mass, fasting, and post meal blood glucose levels. Even synergistically (ACE + Apo E), these two polymorphisms showed no significant association with obesity, fat mass, and blood glucose level. ACE (I/D), Apo E (Hha I), as well as ACE + Apo E seem to have no significant association with obesity, fat mass, and blood glucose levels in this population.

Key words: Angiotensin converting enzyme gene, Apo E gene, Asian Indians, cardiovascular disease, metabolic syndrome, obesity

Introduction

Cardiovascular disease (CVD) is a major public health problem both in developed and developing countries including India. However, its precise etiology and mechanisms remain incompletely understood.[1] Throughout the Asia-Pacific region, there are differences in obesity and body fat distribution, e.g. in Asian population, morbidity and mortality from CVD is occurring in people with lower body mass index (BMI) and smaller waist circumference (WC). Thus, they tend to accumulate intra-abdominal visceral fat without developing generalized obesity. Generalized and central obesity are just two of the interrelated risk factors associated with the cardiometabolic risk factors, e.g. type 2 diabetes mellitus (T2DM).[2] The cardinal question centering the prognosis of CVD and T2DM is whether there exists a single etiological factor clustering in this region. Principal component factor analyses suggested that there exists no single or central etiological factor, rather several underlying abnormalities exist that might have relatively greater genetic basis.[3]

There are several gene polymorphisms known to be associated with CVD and type 2 diabetes. For instance, more than 110 heritable disorders, more than 175 genetic loci, and more than 2050 unique mutations predisposing to stroke are known. The interaction of unfavorable genetic factors, such as the Leiden V, methylenetetrahydrofolate

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reductase (MTHFR) 677TT, apolipoprotein E (Apo E, three different Apo E alleles, namely, e2, e3, and e4 resulting in six different genotypes e2/e2, e3/e3, e4/e4, e2/e3, e2/e4, and e3/e4), and the angiotensin converting enzyme (ACE) gene (deletion/deletion) genotypes (or ACE-D/D), which alone are not major risk factors, in specific patterns exerts a synergistic (combination of different genetic polymorphisms) effect on certain clinical risk factors.[4] Therefore, it is hard to know which polymorphisms are actually involved in the ultimate predisposition of a complex disease.

In a study on ischemic heart disease (IHD), there was no evidence of synergistic effect for the ACE and Apo E genotypes on the risk of early onset IHD.[5] In India, it has been found that ACE gene polymorphism, but not Apo E gene polymorphism, is positively associated with T2DM; however, the synergistic effects of D/D-e3/e3 and I (insertion)/D-e2/e3 on T2DM are also evident.[6] It was also observed that that there lies a significant association between ACE (insertion/deletion or I/D) polymorphisms and hypertension,[7] as well as between Apo E (Hha I) polymorphisms and dyslipidemia.[8] So far as India is concerned, information on the synergistic effects of ACE and Apo E gene polymorphism upon other cardiometabolic risk factors such as obesity, fat mass (FM), and blood glucose is virtually absent.

Keeping this view in mind, the present investigation was aimed to study the synergistic effects of ACE and Apo E gene polymorphism on obesity, FM, and blood glucose among the adult Asian Indians.

Materials and Methods

Study population

The data were originally collected from 350 (184 males and 166 females) adult (aged >30 years) individuals of Calcutta and suburb. Out of 350 subjects, a sample of 139 individuals was selected randomly for genotyping. The institutional ethics committee (IEC) of the Human Genetic Engineering Research Center (HGERC), Calcutta, India, had approved the study. Written consent from participants was also obtained prior to actual commencement of the study.

Anthropometric measures

Anthropometric variables, namely height, weight, and WC, were taken using standard techniques.[9] Height and weight were measured to the nearest 0.1 cm and 0.5 kg, respectively. WC was measured to the nearest 0.1 cm using an inelastic tape. The BMI in kg/m^2 was calculated accordingly, and FM (in kg) was measured using a portable body fat analyzer (Omron Corporation, Tokyo, Japan).

Blood glucose

Both overnight (~12 hours) fasting blood glucose (FBG) and postprandial blood glucose (PPBG) (2 h after habitual meal) were measured by means of a semi auto-analyzer.

Genotyping

DNA was extracted from blood using QI Aamp kit (Qiagen, Hilden, Germany). Genomic DNA was amplified using the polymerase chain reaction with a thermal cycler (Bio Rad, Hercules, California, USA) by specific oligonucleotide primers for ACE[10] and Apo E.[11] I/D polymorphism of ACE was determined by 1.5% agarose gel electrophoresis with ethidium bromide staining. To determine the Apo E polymorphisms, the amplified fragments were digested with "Hha I" restriction enzyme at 37°C overnight, followed by separation using 4% agarose gel electrophoresis with ethidium bromide staining and visualized under UV spectrophotometer.

Statistical analyses

Descriptive statistics such as mean and standard deviation (SD) was calculated for BMI, WC, FM, FBG, and PPBG. The analysis of variance (ANOVA) was undertaken to see the mean difference of risk variables with respect to ACE, Apo E as well as with ACE + Apo E (synergistically) genotypes.

All statistical analyses were computed using SPSS (PC + version 10.0). A *P* value of 0.05 (two tailed) was considered as significant.

Results

The one-way ANOVA for BMI, WC, FM, FBG, and PPBG with respect to ACE I/D polymorphisms is presented in Table 1. There was no significant association for individuals having I/I, I/D, and D/D genotypes with BMI ($P = 0.553$), WC ($P = 0.185$), FM ($P = 0.679$), FBG ($P = 0.104$), and PPBG ($P = 0.311$).

The distribution of risk variables by Apo E genotypes is presented in Table 2. The one-way ANOVA revealed no significant differences between Apo E (Hha I) polymorphisms and BMI ($P = 0.916$), WC ($P = 0.954$), FM ($P = 0.679$), FBG ($P = 0.760$), and PPBG ($P = 0.914$).

The synergistic effect of ACE and Apo E gene polymorphism is presented in Table 3. It was found that there existed no significant differences in the synergistic (additive) effects of ACE + Apo E genotypes on BMI ($P = 0.446$), WC ($P = 0.335$), FM ($P = 0.798$), FBG ($P = 0.234$), and PPBG ($P = 0.489$).

Table 1: Distribution of risk variables by angiotensin converting enzyme (I/D) genotypes

	ACE genotypes (*N* = 139)			
Variables	**I/I (*n* = 46)**	**I/D (*n* = 52)**	**D/D (*n* = 41)**	***P* value for ANOVA**
BMI	21.85 (4.75)	22.30 (4.39)	22.84 (3.39)	0.553
WC	86.65 (12.40)	87.96 (10.38)	90.80 (8.65)	0.185
FM	15.93 (7.03)	16.67 (6.41)	17.10 (5.10)	0.679
FBG	91.54 (19.76)	88.37 (11.77)	97.85 (30.47)	0.104
PPBG (*n* = 105)	130.83 (56.80) (*n* = 35)	117.27 (32.94) (*n* = 37)	135.15 (60.91) (*n* = 33)	0.311

BMI: Body mass index, WC: Waist circumference, FM: Fat mass, FBG: Fasting blood glucose; PPBG: Postprandial blood glucose, Values are mean (±SD)

Table 2: Distribution of risk variables by Apo E (Hha I) genotypes

	Apo E genotypes (*N* = 139)					
Variables	**e2/e3 (*n* = 17)**	**e2/e4 (*n* = 4)**	**e3/e3 (*n* = 91)**	**e3/e4 (*n* = 23)**	**e4/e4 (*n* = 4)**	***P* value for ANOVA**
BMI	22.12 (5.62)	21.67 (3.09)	22.55 (4.17)	21.66 (3.65)	22.00 (4.74)	0.916
WC	88.41 (14.51)	85.50 (7.59)	88.36 (10.38)	88.26 (9.43)	91.75 (13.33)	0.954
FM	16.77 (7.69)	15.42 (2.60)	16.67 (6.18)	16.27 (6.05)	15.57 (7.40)	0.679
FBG	91.06 (23.34)	78.50 (3.70)	92.49 (22.25)	94.22 (18.28)	93.00 (27.82)	0.760
PPBG (*n* = 105)	132.91 (66.04) (*n* = 11)	101.33 (7.02) (*n* = 3)	126.70 (52.08) (*n* = 70)	130.44 (45.26) (*n* = 18)	131.67 (51.87) (*n* = 3)	0.914

Values are mean (±SD)

Table 3: Distribution of risk variables by synergistic effects of angiotensin converting enzyme (I/D) and Apo E (Hha I) genotypes

	Synergistic (*N* = 139)							
Variables	**1 (*n* = 5)**	**2 (*n* = 11)**	**3 (*n* =35)**	**4 (*n* = 40)**	**5 (*n* = 38)**	**6 (*n* = 8)**	**7 (*n* = 2)**	***P* value for ANOVA**
BMI	19.8 (4.73)	22.11 (4.94)	22.65 (5.14)	21.97 (4.43)	23.09 (2.77)	20.16 (3.66)	24.00 (1.27)	0.446
WC	83.40 (16.15)	88.00 (13.43)	88.06 (12.42)	86.80 (9.80)	91.13 (8.56)	85.50 (8.09)	98.50 (3.54)	0.335
FM	13.90 (7.69)	16.80 (7.38)	16.89 (7.08)	16.19 (6.36)	17.13 (5.06)	14.45 (5.80)	20.40 (4.52)	0.798
FBG	81.80 (20.24)	87.91 (9.46)	91.69 (21.50)	89.50 (14.07)	99.76 (29.84)	88.13 (12.29)	78.50 (13.44)	0.234
PPBG	128.00 (24.71) (*n* = 3)	108.33 (3.50) (*n* = 6)	120.40 (41.65) (*n* = 30)	125.71 (47.46) (*n* = 28)	139.17 (62.15) (*n* = 30)	118.50 (29.76) (*n* = 6)	103.00 (21.21) (*n* = 2)	0.489

Genotypes combination: (1) e2/e3-I/I; (2) e2/e4-I/D and e2/e3-I/D; (3) e3/e3-I/I and e2/e4-I/D and e2/e3-D/D; (4) e3/e4-I/I and e3/e3-I/D; (5) e4/e4-I/I and e3/e4-I/D and e3/e3-D/D; (6) e4/e4-I/D and e3/e4-D/D; (7) e4/e4-D/D; Values are mean (±SD)

Discussion

The present investigation was aimed to examine whether there exists any significant association of ACE (I/D) and Apo E (Hha I) polymorphisms with BMI, WC, FM, FBG, and PPBG, either independently or synergistically. It was found that neither ACE (I/D) nor Apo E (Hha I) gene polymorphism had significant association with BMI, WC, FM, FBG, and PPBG. Moreover, synergistically also, ACE (I/D) and Apo E (Hha I) had no significant association with BMI, WC, FM, FBG, and PPBG. However, significant association of ACE I/D with hypertension[7] and Apo E (Hha I) with dyslipidemia[8] was observed in the same studied population.

Therefore, it seems reasonable to argue that these polymorphisms (either independently or synergistically) apparently do not have significant association with individual risk variables often considered to describe cardiometabolic syndrome. However, whenever constellation of risk variables is arguing, these polymorphisms do play significant role(s) in the manifestation of many chronic diseases (e.g. dyslipidemia, metabolic syndrome, etc.).[3,7,8]

However, the major limitation of the study was that it was performed on a relatively small sample size. Therefore, further study on large sample size from different parts of India would give better insight into proper understanding of the synergistic effects of these gene polymorphisms on the CVD events in the people of South Asian descent.

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